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## MICROBIOLOGY

# Walls talk: Microbial biogeography of homes spanning urbanization

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Westernization has propelled changes in urbanization and architecture, altering our exposure to the outdoor environment from that experienced during most of human evolution. These changes might affect the developmental exposure of infants to bacteria, immune development, and human microbiome diversity. Contemporary urban humans spend most of their time indoors, and little is known about the microbes associated with different designs of the built environment and their interaction with the human immune system. This study addresses the associations between architectural design and the microbial biogeography of households across a gradient of urbanization in South America. Urbanization was associated with households' increased isolation from outdoor environments, with additional indoor space isolation by walls. Microbes from house walls and floors segregate by location, and urban indoor walls contain human bacterial markers of space use. Urbanized spaces uniquely increase the content of human-associated microbes—which could increase transmission of potential pathogens—and decrease exposure to the environmental microbes with which humans have coevolved.

## INTRODUCTION

Urbanization of traditional villages—the villages developing in more urban form, and historical villagers migrating to towns and cities—is occurring concurrently with a global convergence toward a more Westernized urban plan and life-style (1). This process occurs as human societies integrate from hunter-gatherers into first rural and then urban life-styles. Urbanization also involves more people spending most of their lives in indoor built environments (2, 3).

A large proportion of the microbes found in the built environment are shed by humans (4–7) or animals (8), and with natural ventilation, microbes can also be transported from outdoors (5, 6, 9). Understanding the consequences of architectural changes on environmental exposures, including microbial exposures, is therefore important in improving home design and ultimately human health. Here, we determine the changes in architectural design and the resulting microbial communities of houses spanning a range of modernization within the Amazon River basin. We measured community demographics and architectural parameters, and characterized the microbial communities of 10 houses and their inhabitants from each of four locations: a traditional jungle village of hunter-gatherers near the border between Peru and Ecuador, a rural village further east along a similar latitude, the large Peruvian town of Iquitos, and, finally, the modern Brazilian city of Manaus (Fig. 1A).

## RESULTS

The jungle village of Checherta is a 21-house Achuar community of hunter-gatherers (tables S1 and S2, and fig. S1, A and B). Homes are organized around a central area, including a communal building. This community design is retained in the 25-house rural village of Puerto Almendras, with the homes surrounding a soccer field (fig. S1C and table S1). Iquitos has 0.4 million inhabitants and is the largest urban population in the world not accessible by roads (fig. S1C and tables S1 and S2). Manaus, the capital of Amazonas State in Brazil, is a contemporary Western city with 1.8 million inhabitants (fig. S1D and tables S1 and S2).

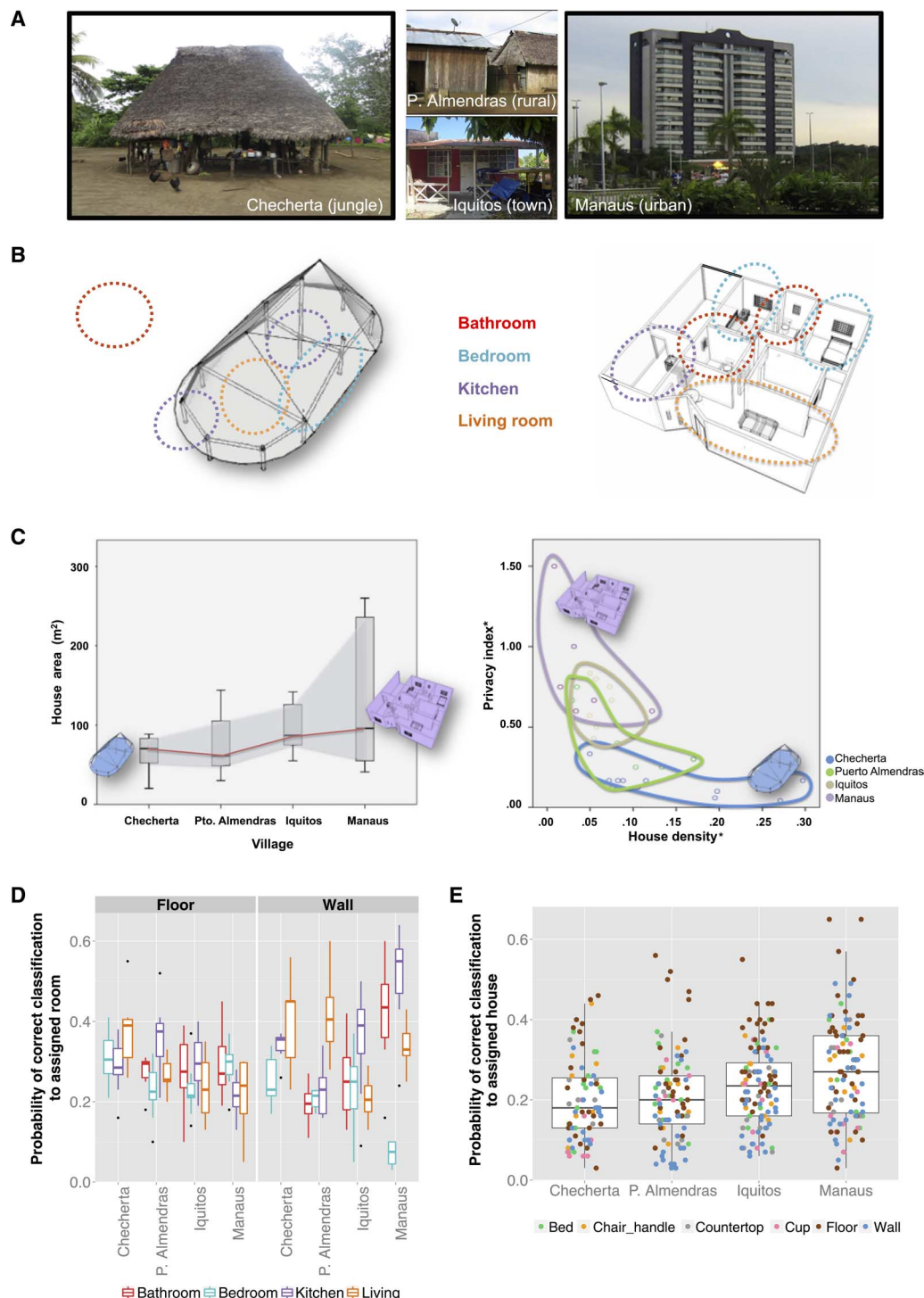
Although no significant environmental differences were found across the urbanization gradient, large architectural changes were observed (Fig. 1). No significant differences were found across the studied locations in outdoor temperature (mean variation, <2°C; table S3) or relative humidity, and all locations had high ventilation rates (air exchange rates of 25 to 100 h<sup>-1</sup> in the jungle village, 7 to 20 h<sup>-1</sup> in the rural village, 4 to 17 h<sup>-1</sup> in the town, and 0.8 to 15 h<sup>-1</sup> in the city). The jungle village homes of Checherta are open huts made of wood and reeds, and are generally single open-plan spaces composed of two functional areas (Fig. 1, A and B, and fig. S2): a dormitory containing one platform bed per family, and a fire area for cooking and socializing. Up to six core families, among extended family members, share a home. As urbanization increases, a progressive separation of the indoor environment from the outdoor occurs first, followed by internal division of home spaces and the use of a wider variety of building materials (table S4). In the rural village, a toilet appears as an external latrine, which in the town and city becomes a piped indoor bathroom. Town and city houses typically have additional spaces differentiated by functional purpose (living room, kitchen, and bathroom) and segregated by walls (Fig. 1B).

Houses in the most urbanized conditions are more variable in design, but in general, there is an urbanization-associated increase in the number of rooms per person (privacy index) (Fig. 1C, fig. S3, and table S4), house area, and its variance ( $P < 0.005$ ; Fig. 1C and table S4). The average

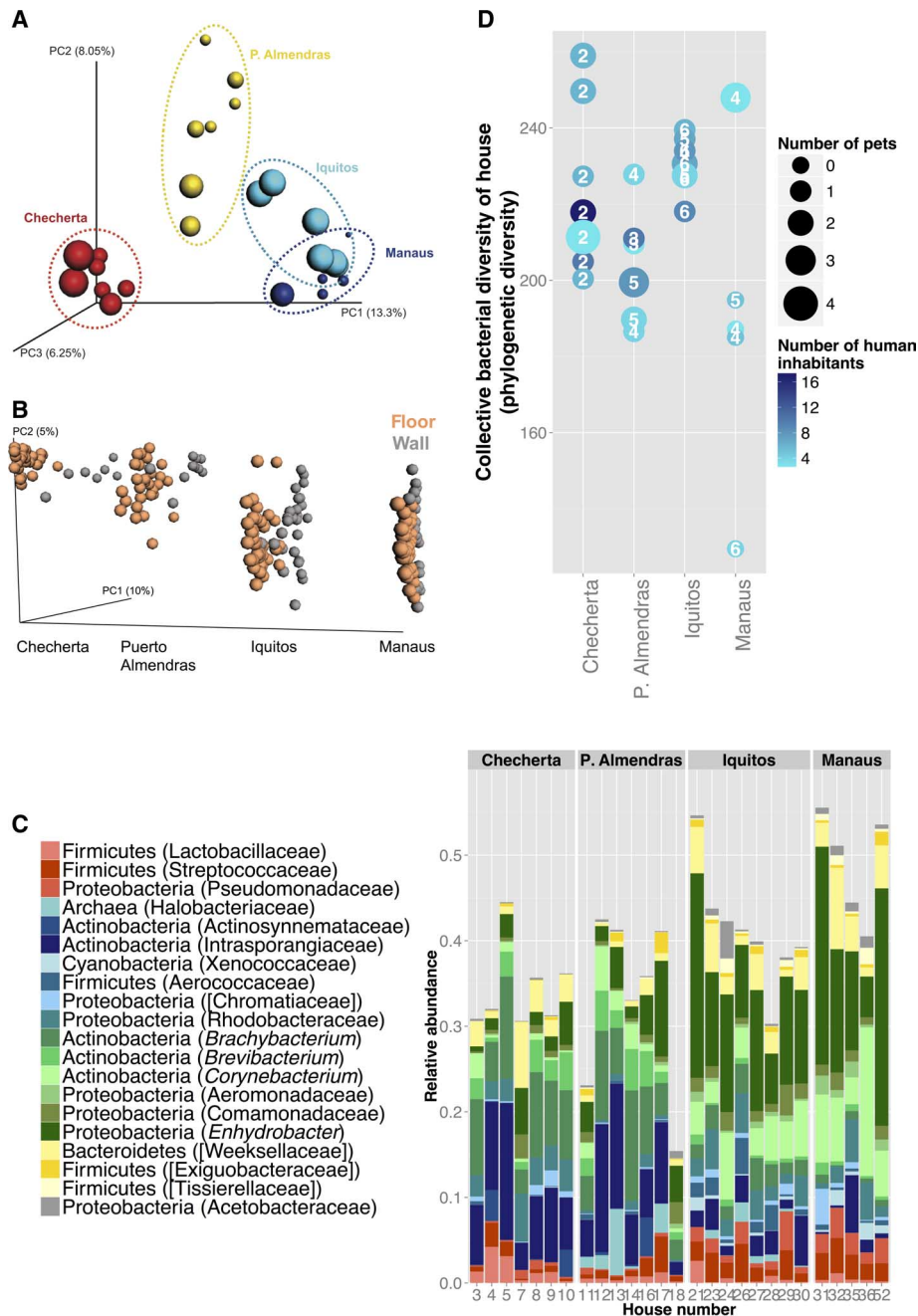
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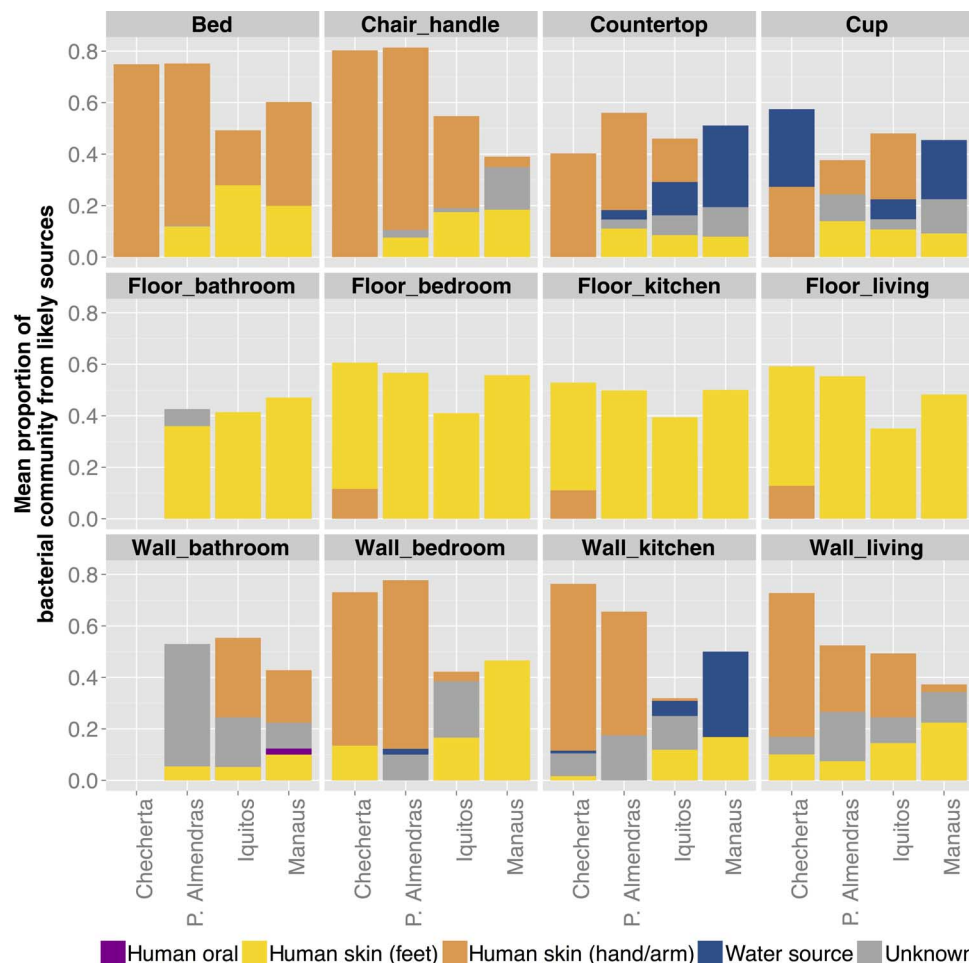
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**Fig. 1. Architecture and space use covary with key structural features such as house partitioning, area, and occupant density across the four locations; differences in space use are reflected in the microbial communities of the walls, but not floors, which contribute to the microbial signatures of the homes.** (A) Photos of the typical structures found across the four communities along this urbanization gradient [Checherta (jungle), Puerto Almendras (rural), Iquitos (town), and Manaus (city)]. (B) Typical floor plans of houses in Checherta and Manaus (left and right, respectively). (C) Distribution of house area (left) and mean privacy index\* (privacy index = number of rooms/number of people) according to occupant density (occupant density = number of people/square meters) (right) by location. (D) Classification probability of correct assignment to a sample's true location using a random forest classifier. The probability of being able to predict a functional space using the microbial community of the walls increases with increased partitioning of spaces by use in the urban areas (for example, bathrooms and kitchens in separate walled-off spaces). Floor microbial communities, on the other hand, are not as discriminatory among rooms. (E) Classification probability of correct assignment of a given sample to the correct house.



**Fig. 2. Microbial community structure in houses differs significantly across the urbanization gradient.** Seven sites that were common to all houses (living room, bedroom, kitchen floors, beds, chair handles, countertops, and living room walls) were collapsed into one sample to obtain a total measure of diversity for each home. **(A)** Principal coordinates analysis (PCoA) of the seven collapsed samples for each home shows tight clustering of the samples by community ( $P < 0.01$ , analysis of similarities). Point size shows the  $\alpha$  diversity level, measured as phylogenetic diversity (PD) (smallest, <150; largest, >250). **(B)** PCoA plot of unweighted UniFrac distances of wall and floor bacterial communities by village. Floor samples are clustered very tightly in the jungle community, but not wall samples. This indicates that floor microbial communities resemble more to each other than wall samples. This clustering of floor samples decreases with urbanization, and microbial communities of walls and floors merge in urban locations, meaning that urban locations have similar microbes on the walls and floors, whereas in rural locations, floors have very different microbial communities. **(C)** Top 20 feature taxa of high relative abundance (>0.1%) that allowed for correct prediction of a sample's source community; these include taxa commonly associated with humans (for example, Streptococcaceae, Lactobacillaceae, and Pseudomonadaceae) (shown in red hues) and taxa commonly associated with the environment (for example, Intrasporangiaceae and Rhodobacteraceae) (shown in blue hues). Taxa shown in the literature to be associated with both the environment and the human body are shown in green hues. **(D)** Distribution of the collective  $\alpha$  diversity (PD) of each home, colored by the number of human inhabitants residing in the home. Numbers inside the points indicate the number of different material types that are represented by the seven samples, and the size corresponds to the total number of pets in the home (dog, cat, monkey, chicken, turtle, or parrot).



**Fig. 3. Source tracking indicates home sample's bacteria reflecting typical use.** For each home, the human oral and skin samples as well as a water source, such as a water bucket or faucet, were input as potential sources of microbes found in sites around the home. Values shown represent likely contributions from each of these sources, averaged across the homes in each community. All sites contain at least a considerable proportion of taxa that are also found on the skin of the home's inhabitants, with floors showing the highest levels of similarity.

house occupancy (persons per square meter) decreases with urbanization ( $P < 0.005$ ; Fig. 1C and table S4), which is consistent with higher area and smaller families.

Remarkably, classification of house functional spaces using microbes was possible (Fig. 1D and fig. S4), and the probability of correct assignment given the wall bacterial composition increased with urbanization (Fig. 1E). We tested for differences in the types and diversity of household bacteria across locations. Microbial richness ( $\alpha$  diversity) did not change with urbanization (Fig. 2 and figs. S5 and S6), but bacterial composition was markedly different (Fig. 2, A and B, and figs. S7 and S8) with houses becoming more microbially distinct along the gradient. Bacterial community structure in samples from floor and walls converged with urbanization (Fig. 2B). At the jungle end of the gradient, floors were made of dirt and people walked barefoot, and walls were wood columns; at the city end, floors and walls were made of synthetic materials, and people walked with shoes (in all but one house). Moreover, wall microbes better differentiated the kitchen and bathroom functional spaces in urban than in rural houses (Fig. 1D and fig. S9). The 10 most important operational taxonomic units (OTUs) that help discriminate among rooms in Manaus com-

prise several taxa normally associated with the human oral cavity, including *Streptococcus*, *Neisseria*, *Actinomyces*, and *Veillonella dispar*, as well as taxa normally associated with the human gut such as *Enterobacteriaceae*.

Despite lower occupant density in urban houses, “humanization” of the houses occurred with increased urbanization (Fig. 2D), associated with home enclosure—isolation from the outdoor environment—especially in dwellings sealed for air conditioning. Human bacteria were enriched in the town and city houses, with *Prevotella*, *Verrucomicrobia*, and *Serratia* on the walls (figs. S7 and S10), and skin taxa on the floors, consistent with human shedding (7, 10–12) and with the isolation of homes from bacterial sources from outdoor environments. Environmental bacteria were proportionally higher in the jungle and rural village house floors and included soil bacteria [for example, *Mesorhizobium* and *Luteimonas* from water sources and *Rickettsiella* from arthropods (figs. S7 and S10)]. The environmental bacterium found in walls included *Acidobacteriales*, *Bradyrhizobium*, *Dactylosporangium*, *Actinomycetospora*, *Actinoalloteichus*, *Saccharopolyspora*, *Pedomicrobium*, and *Rickettsiella* (figs. S7 and S10). As we move from the rural to the urban locations, there is a shift within Actinobacteria,



from *Brachybacterium* and *Brevibacterium* commonly found in the environment to *Corynebacterium*, common in human skin (Fig. 2B).

A Bayesian approach called SourceTracker allowed the estimation of proportion of each community (that is, sample) that are likely to originate from each of a specified set of source environments (9). This analysis further confirmed the presence of a partially oral-like community on the urban bathroom walls (Fig. 3); these traces of human oral microbes from bathrooms and traces of water-associated microbes on kitchen countertops and walls likely contribute to the increased ability to identify both the houses and the indoor functional spaces.

We found no systematic association between the bacterial communities and many other parameters measured in the study including the structural materials in the households, number of people living in the house, number of pets (Fig. 2C), temperature variations, light incidence, frequency of cleaning, number of outsiders at sampling time, date of last rain, and time of day samples were collected ( $P > 0.05$  in all cases). In particular, consistent with recent studies (11, 13), we find that samples within a house with different materials are more similar to one another than samples from the same material across different houses and that, in all communities, the inhabitants of each house are a major source of bacteria (Fig. 3).

## DISCUSSION

Our findings indicate that the bacteria from the surfaces of house walls are informative of level of urbanization based on architectural design. Floors are the most informative of the commonalities found in individual houses across urbanization levels, whereas walls, less perturbed reservoirs of microbes accumulated through room usage, provide an indicator of room function.

Ventilation, described as a key factor for microbial community composition in urban settings (14–16), was very high in all of the houses of our study and does not explain differences in home microbial composition with urbanization. Instead, we propose that the presence of walls dividing functional spaces acquires function-dependent microbes, mostly of human origin.

The current study is limited to one geographical region of the world and is a small pilot study, and thus, results may not be generalizable. Further research should identify mechanistic explanations for these phenomena. Insights into the chemical signals that bacteria provide in different sites within the home are also needed. These remarkable changes in house microbial content across urbanization might translate into differences in microbial exposure that may have developmental health implications for humans (17), according to several related hypotheses [the “hygiene” hypothesis (18), the “Old Friends” hypothesis (19), and the “Disappearing microbiota” hypothesis (20)], suggesting that the reduced pattern of microbial exposure leads to immune and metabolic disorders that have become the new disease paradigm in the industrialized world.

## MATERIALS AND METHODS

### Design of the study

We selected four communities at the same latitude in the Amazon Basin, with different degrees of urbanization (fig. S1): an isolated jungle village, a rural community, an urban town, and an urban city.

The specific locations were selected to represent four significantly different urbanization levels with similar climate. Ten houses from each location were sampled in four sites to characterize architectural and microbiological profiles of house walls and floors. The sample size of  $n = 40$  per location was based on estimations using a two-sided test, for significant differences in the microbial composition, with a Cohen’s  $d = 0.63$ , power of 80%, and  $\alpha = 0.05$ .

### Communities’ description

Four human settings were studied in this work, spanning urbanization. Three of them were in Peru, and one in Brazil.

The Peruvian rural community of Checherta is a traditional, native, hunter-gatherer, Amerindian village in the border between Peru and Ecuador (fig. S1). It is inhabited by approximately 300 inhabitants, living in open huts, with the exception of one house that was enclosed from the outside by walls (fig. S11), made of natural materials (Fig. 1A and fig. S2). It has a recently made school consisting of one classroom and three adjacent latrines, which remain unused by the locals. Checherta has no electricity or potable water services; water is obtained from the nearest river, and the village is highly inaccessible, requiring travel by a plane that can land on an improvised landing field in Nuevo Andoas and then taking a 2-day trip on small river boats (table S1).

The second Peruvian community, Puerto Almendras, is a rural setting located at ~1-hour drive (12 km) west from Iquitos. It has ~250 inhabitants that live in houses with external walls, made out of both natural and industrial materials. Most of the houses were not internally subdivided, and those spaces that were remained connected with adjacent areas because the walls did not reach the roof. Puerto Almendras has a water reservoir (however, no potable water service), electricity service, a school, and a health care center within walking distance. Houses are distributed around a soccer field.

The third Peruvian community was the town of Iquitos, the world’s biggest populated center that is inaccessible by road—it is accessible only by plane or boat (table S1). This town has 371,000 inhabitants, an international airport, paved roads, municipally treated piped water, and electricity. All houses are enclosed in external walls that separate them from the outdoor environment and are made of industrial materials. Walls that divide the inner house do not always reach the roof.

The fourth location was Manaus in Brazil, the biggest city in the Amazon region, with a population of 1.8 million, accessible by roads, boats, and planes. Sampled houses were completely separated from the environment and internally divided by walls. Unlike the Peruvian communities, this city has enormous social differences, and we sampled homes from middle class families.

### Architectural determinations

Sketches of the houses were created with measurements collected in the field, with photographs of each household (Fig. 1A and figs. S2 and S11), that provided the basis for estimations of floor area/surface, volume, openness (proportion of apertures, location in floor plans, and orientation), human density (number of people per square meter), and privacy index for each household. Additionally, this information sets the basis for modeling using a building modeling program (Autodesk Revit) to produce three-dimensional representations of each sampled house.

Environmental variables of temperature and relative humidity were collected. A HOBO Micro Station Data Logger (H21-002) was used to record 2-min interval data of temperature and relative humidity.

Analysis of qualitative data from both architecture and environmental variables was made using the SPSS version 20 program to compare variations in architecture and environment between locations.

### Bacterial community structure determinations

Microbial samples were collected (using sterile swabs) from floors and walls of living rooms, kitchens, bedrooms, and bathrooms—or equivalent functional spaces in jungle houses—of each household. Metadata information from each sample was recorded, including surface material, sample height (walls), cleaning frequency, presence of pets in the home, light, surface temperature, and whether people wore shoes. Shoes were worn in 36% of family members in Checherta, 75% in Puerto Almendras, and 100% in Iquitos and Manaus.

Cryovial-containing samples were frozen in a dry shipper and stored at  $-80^{\circ}\text{C}$  until DNA was extracted using the MoBio PowerSoil Kit (following the manufacturer's instructions). The V3-V4 regions of the 16S *rRNA* gene were sequenced using the HiSeq Illumina platform. Sequences were analyzed using the Qiime pipeline. Sequences were trimmed at 100 base pairs, and open-reference OTU picking (21) was performed at a 97% identity to assign taxonomy using Greengenes version 13.8 (22) and to characterize novel taxa.  $\alpha$  Diversity was estimated using PD whole tree (23) on rarefied tables at 10,000 sequences per sample for floors and 2500 sequences per sample for walls.  $\beta$  Diversity was measured using unweighted UniFrac (24) on the rarefied tables. Finally, the Bayesian approach SourceTracker was used to identify possible sources of contamination (9).

### SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/2/2/e1501061/DC1>

Fig. S1. Satellite view and urban plan of the communities selected for this study in the South American Amazonas.

Fig. S2. Typical house in the jungle community of Checherta.

Fig. S3. Distribution of privacy index on each location.

Fig. S4. UniFrac-based PCoA of microbial communities in the floors (A and B) and walls (C and D), colored by village (A and C) and by room (B and D).

Fig. S5. Bacterial  $\alpha$  diversity calculated with the PD whole tree metric on floor and wall samples by location.

Fig. S6. Bacterial  $\alpha$  diversity calculated with the PD whole tree metric on floor and wall samples by home site in each location.

Fig. S7. Taxonomic composition of floor and wall samples from each location.

Fig. S8. UniFrac distances of the microbial communities in floors and walls, and between and within locations.

Fig. S9. UniFrac-based PCoA of microbial communities in the walls of jungle (Checherta) and city (Manaus).

Fig. S10. Discriminative bacteria from each location based on an LDA effective size (LEfSe) analysis on floor and walls from all villages.

Fig. S11. Blueprint of a closed, segregated house from Checherta.

Table S1. Urban parameters of the four sampling sites for this study.

Table S2. Architectural qualitative descriptions of the studied settlements.

Table S3. Summary statistics for indoor temperature and relative humidity on the four locations at 1-min intervals.

Table S4. Architectural parameters for the studied settlements (averages and SDs).

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